



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 31/66, 31/19	A1	(11) International Publication Number: WO 00/21542 (43) International Publication Date: 20 April 2000 (20.04.00)
(21) International Application Number: PCT/US99/23757 (22) International Filing Date: 12 October 1999 (12.10.99) (30) Priority Data: 60/104,374 15 October 1998 (15.10.98) US 9824573.1 9 November 1998 (09.11.98) GB (71) Applicants (for all designated States except US): MERCK & CO., INC. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). MERCK FROSST CANADA & CO. [CA/CA]; 16711 Trans-Canada Highway, Kirkland, Québec H9H 3L1 (CA). (72) Inventors; and (75) Inventors/Applicants (for US only): HARADA, Shun-Ichi [JP/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). MACHWATE, Mohamed [MA/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). RODAN, Gideon, A. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). WEINREB, Miron [IL/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). LABELLE, Marc [CA/CA]; 16711 Trans-Canada Highway, Kirkland, Québec H9H 3L1 (CA). METTERS, Kathleen [CA/CA]; 16711 Trans-Canada Highway, Kirkland, Québec H9H 3L1 (CA). YOUNG, Robert, N.		[CA/CA]; 16711 Trans-Canada Highway, Kirkland, Québec H9H 3L1 (CA). (74) Common Representative: MERCK & CO., INC.; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). (81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: METHODS FOR STIMULATING BONE FORMATION (57) Abstract <p>The present invention relates to methods for stimulating bone formation in a mammal comprising administering to a mammal in need thereof a therapeutically effective amount of an EP₄ receptor subtype agonist.</p>		

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TITLE OF THE INVENTION
METHODS FOR STIMULATING BONE FORMATION

CROSS REFERENCE TO RELATED APPLICATIONS

- 5 The present application claims priority of U.S. provisional application
Serial No. 60/104,374, filed October 15, 1998.

BRIEF DESCRIPTION OF THE INVENTION

- 10 The present invention relates to methods for stimulating bone
formation, i.e. osteogenesis, in a mammal comprising administering to a mammal in
need thereof a therapeutically effective amount of an EP₄ receptor subtype agonist.

BACKGROUND OF THE INVENTION

- 15 A variety of disorders in humans and other mammals involve or are
associated with abnormal or excessive bone loss. Such disorders include, but are not
limited to, osteoporosis, glucocorticoid induced osteoporosis, Paget's disease,
abnormally increased bone turnover, periodontal disease, tooth loss, bone fractures,
rheumatoid arthritis, periprosthetic osteolysis, osteogenesis imperfecta, metastatic
bone disease, hypercalcemia of malignancy, and multiple myeloma. One of the most
20 common of these disorders is osteoporosis, which in its most frequent manifestation
occurs in postmenopausal women. Osteoporosis is a systemic skeletal disease
characterized by a low bone mass and microarchitectural deterioration of bone tissue,
with a consequent increase in bone fragility and susceptibility to fracture.
Osteoporotic fractures are a major cause of morbidity and mortality in the elderly
25 population. As many as 50% of women and a third of men will experience an
osteoporotic fracture. A large segment of the older population already has low bone
density and a high risk of fractures. There is a significant need to both prevent and
treat osteoporosis and other conditions associated with bone resorption. Because
osteoporosis, as well as other disorders associated with bone loss, are generally
30 chronic conditions, it is believed that appropriate therapy will typically require chronic
treatment.

- Normal bone physiology involves a process wherein bone tissue is
continuously being turned over by the processes of modeling and remodeling. In
other words, there is normally an appropriate balance between resorption of existing
35 bone tissue and the formation of new bone tissue. The exact mechanism underlying

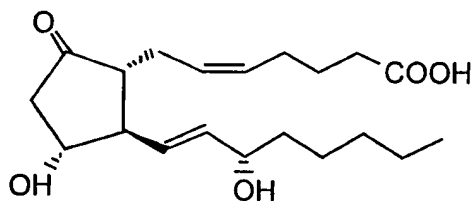
the coupling between bone resorption and formation is still unknown. However, an imbalance in these processes is manifested in various disease states and conditions of the skeleton.

Two different types of cells called osteoblasts and osteoclasts are involved in the bone formation and resorption processes, respectively. See H. Fleisch, *Bisphosphonates In Bone Disease, From The Laboratory To The Patient*, 3rd Edition, Parthenon Publishing (1997), which is incorporated by reference herein in its entirety.

Osteoblasts are cells that are located on the bone surface. These cells secrete an osseous organic matrix, which then calcifies. Substances such as fluoride, parathyroid hormone, and certain cytokines such as prostaglandins are known to provide a stimulatory effect on osteoblast cells. However, an aim of current research is to develop therapeutic agents that will selectively increase or stimulate the bone formation activity of the osteoblasts.

Osteoclasts are usually large multinucleated cells that are situated either on the surface of the cortical or trabecular bone or within the cortical bone. The osteoclasts resorb bone in a closed, sealed-off microenvironment located between the cell and the bone. The recruitment and activity of osteoclasts is known to be influenced by a series of cytokines and hormones. It is well known that bisphosphonates are selective inhibitors of osteoclastic bone resorption, making these compounds important therapeutic agents in the treatment or prevention of a variety of systemic or localized bone disorders caused by or associated with abnormal bone resorption. However, despite the utility of bisphosphonates there remains the desire amongst researchers to develop additional therapeutic agents for inhibiting the bone resorption activity of osteoclasts.

Prostaglandins are alicyclic compounds related to the basic compound prostanoic acid. A natural prostaglandin, PGE₂, has the following structure.



Prostaglandins such as PGE₂ are known to stimulate bone formation and increase bone mass in mammals, including man. It is believed that four different receptor subtypes, designated EP₁, EP₂, EP₃, and EP₄ are involved in mediating the bone modeling and remodeling processes of the osteoblasts and osteoclasts. The major prostaglandin receptor in bone is EP₄, which is believed to provide its effect by signaling via cyclic AMP. However, the scientific information that is currently known about the prostaglandin mediated bone effect is rather limited, because the exact mechanism of action is not known. Prostaglandins and their accosted receptors are more fully described in for example, K. Ono et al., *Important role of EP₄, a subtype of prostaglandin (PG) E receptor, in osteoclast-like cell formation from mouse bone marrow cells induced by PGE₂*, *J. of Endocrinology*, 158, R1-R5 (1998), C.D. Funk et al., *Cloning and Expression of a cDNA for the Human Prostaglandin E Receptor EP₁ Subtype*, *Journal of Biological Chemistry*, vol. 268, no. 35, pp. 26767-26772 (1993), J.W. Reagan et al., *Cloning of a Novel Human Prostaglandin Receptor with Characteristics of the Pharmacologically Defined EP₂ Subtype*, *Molecular Pharmacology*, vol. 46, pp. 213-220 (1994), J. Yang et al., *Cloning and Expression of the EP₃-Subtype of Human Receptors for Prostaglandin E₂*, *Biochemical Biophysical Research Communication*, vol., 198, pp. 999-1006 (1994), L. Bastien et al., *Cloning, Functional Expression and Characterization of the Human Prostaglandin E₂ Receptor EP₂ Subtype*, *Journal Biological Chemistry*, vol. 269, pp. 11873-11877 (1994), which are all incorporated by reference herein in their entirety.

In the present invention it is found that agonists of the EP₄ subtype receptor are useful for stimulating bone formation. Without being limited by theory, it is believed that these agonists are responsible for upregulating the number and/or activity of the osteoblasts.

It is an object of the present invention to provide methods for stimulating bone formation, i.e. osteogenesis, in a mammal comprising administering to a mammal in need thereof a therapeutically effective amount of an EP₄ receptor subtype agonist.

It is another object of the present invention to provide methods for treating or reducing the risk of contracting a disease state or condition in a mammal in need of such treatment or prevention, comprising administering to said mammal a therapeutically effective amount of an EP₄ receptor subtype agonist.

It is another object of the present invention to provide methods for stimulating bone formation in a mammal in need thereof comprising administering to said mammal a therapeutically effective amount of an EP₄ receptor subtype agonist and a bisphosphonate active.

5 It is another object of the present invention to provide pharmaceutical compositions comprising a therapeutically effective amount of an EP₄ receptor subtype agonist.

 It is another object of the present invention to provide pharmaceutical compositions comprising a therapeutically effective amount of an EP₄ receptor
10 subtype agonist and a bisphosphonate active.

 It is another object of the present invention to identify EP₄ receptor subtype agonists useful for stimulating bone formation.

 These and other objects will become readily apparent from the detailed description which follows.

15

SUMMARY OF THE INVENTION

 The present invention relates to methods for stimulating bone formation, i.e. osteogenesis, in a mammal comprising administering to a mammal in need thereof a therapeutically effective amount of an EP₄ receptor subtype agonist.

20 In further embodiments, the present invention relates to methods for treating or reducing the risk of contracting a disease state or condition involving bone tissue in a mammal in need of such treatment or risk reduction, comprising administering to said mammal a therapeutically effective amount of an EP₄ receptor subtype agonist.

25 In further embodiments, the present invention relates to methods for stimulating bone formation in a mammal in need thereof comprising administering to said mammal a therapeutically effective amount of an EP₄ receptor subtype agonist and a bisphosphonate active.

30 In further embodiments, the present invention relates to pharmaceutical compositions comprising a therapeutically effective amount of an EP₄ receptor subtype agonist.

 In further embodiments, the present invention relates to pharmaceutical compositions comprising a therapeutically effective amount of an EP₄ receptor subtype agonist and a bisphosphonate active.

In further embodiments, the present invention relates to a method for identifying agonists of an EP₄ receptor subtype.

In further embodiments, the present invention relates to the use of a composition in the manufacture of a medicament for stimulating bone formation, i.e. osteogenesis, in a mammal comprising administering to a mammal in need thereof a therapeutically effective amount of an EP₄ receptor subtype agonist.

All percentages and ratios used herein, unless otherwise indicated, are by weight. The invention hereof can comprise, consist of, or consist essentially of the essential as well as optional ingredients, components, and methods described herein.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to methods for stimulating bone formation, i.e. osteogenesis, in a mammal comprising administering to a mammal in need thereof a therapeutically effective amount of an EP₄ receptor subtype agonist.

Prostaglandins E (especially PGE₂) stimulate bone formation and increase bone mass in several species, including man. The mechanism of this effect, the target cells and the receptors involved are not completely known. Specific cell-surface receptors for PGE₂, EP₁₋₄, which employ distinct secondary messenger systems have been cloned and characterized. It is believed that cyclic AMP may have a role in osteogenesis induced by PGE₂. The expression of the EP₂ and EP₄ receptors, which are coupled to the cyclic AMP pathway, is investigated in the bone tissue of young adult rats (where PGE₂ is markedly anabolic), and in various osteoblastic cell lines. Osteoblastic cell lines, RCT-1, RCT-3, TRAB-11 and RP-1, as well as osteoblastic cells harvested from fetal rat bones express EP₄ mRNA but not EP₂ mRNA. In addition, EP₄ mRNA is expressed in tibiae and calvariae of 5-week-old rats while EP₂ is not.

Treatment of periosteal cells (RP-1) *in vitro* with 10⁻⁶ M PGE₂ increases the level of EP₄ mRNA which peaks at 2 hours. Similarly, systemic administration of an anabolic dose of PGE₂ (3-6 mg/kg) to young adult rats upregulates the expression of EP₄ in tibiae and calvariae, an effect which peaks at 1-2 hours. Using *in-situ* hybridization it is found that the increased expression of EP₄ mRNA in the tibial metaphysis following systemic PGE₂ treatment is localized to bone marrow cells.

EP₄ is expressed in osteoblastic cells *in vitro* and in bone marrow putative osteoprogenitor cells *in vivo* and is upregulated by its ligand, PGE₂. Given

the presence of EP₄ expression in the cells examined and in bone tissue, it is found in the present invention that EP₄ is the receptor subtype which mediates the anabolic effects of PGE₂.

Prostaglandins (especially PGE₂) have multiple effects on bone, stimulating both resorption and formation. Systemic administration of PGE₁ or PGE₂ to infants and to animals is clearly anabolic, stimulating bone formation and increasing bone mass. Also local administration of PGE₂ into long bones stimulates new bone formation, suggesting that PGE₂ acts directly on bone tissue to induce osteogenesis. Histological analysis of bones treated with PGE₂ indicates that PGE₂ increases the number of osteoblasts present on the bone surface, suggesting that prostaglandins act by recruiting osteoblasts from their precursors and/or sustaining existing osteoblasts.

PGEs act on various cells via specific cell-surface receptors divided into 4 subtypes, EP₁₋₄, according to their relative sensitivity to selective agonists and antagonists. The receptor subtypes all belong to the G-protein-coupled receptor family and activate distinct secondary messenger systems such as adenylate cyclase or phospholipase C. Of these 4 receptors, EP₄ and EP₂ activate adenylate cyclase, EP₁ activates phospholipase C, and EP₃ either lowers intracellular cAMP levels or activates phospholipase C, depending on the specific spliced variant.

In osteoblastic cells *in vitro*, PGE₂ stimulates both phosphatidylinositol and cyclic AMP transduction pathways. Both EP₁ and EP₄, found in osteoblastic MC3T3-E₁ cells are believed to play a role in the biological action of PGE₂ in bone tissue. Also PGE₁, a potent inducer of bone formation in humans and other species, increases intracellular cyclic AMP but has no effect on phosphatidylinositol turnover in osteoblastic cells. It is therefore believed that PGE receptors coupled to adenylate cyclases, EP₂ and/or EP₄, are involved in osteogenesis. Initial characterization of *in vivo* expression of EP receptors by *in situ* hybridization shows that in embryonic and neonatal mice, EP₄ is the major form found in bone tissue, especially in preosteoblasts. See Ikeda T, Miyaura C, Ichikawa A, Narumiya S, Yoshiki S and Suda T 1995, *In situ localization of three subtypes (EP₁, EP₃ and EP₄) of prostaglandin E receptors in embryonic and newborn mice.*, *J Bone Miner Res* 10 (sup 1):S172, which is incorporated by reference herein in its entirety.

Also, it is found that EP₄, but not EP₂, mRNA is expressed in adult rat bone tissue and bone-derived cell lines and that expression is stimulated by PGE₂.

Analysis of the *in vivo* expression of PGE receptors shows that EP₄, but not EP₂, is expressed in total RNA from adult rat tibiae and calvariae. EP₄ is
5 believed to be the major adenylate cyclase-coupled PGE₂ receptor expressed in osteoblastic cells and in bone tissue. Also, the EP₄ receptor subtype is expressed in the bone tissue of young adult rats, in which PGE₂ is strongly anabolic.

EP₄ mRNA is expressed in osteoblast precursor cells. It is also found in less differentiated bone cell lines such as RCT-1, TRAB-11 and the RP-1 periosteal
10 cells, but not in fibroblasts. It is highly expressed in bone marrow cells that include osteoblast precursor cells, but not in fully mature osteoblasts on the bone surface. It is believed that PGE₂ induces osteogenesis via an increase in the number of active osteoblasts present on the bone surface, resulting from the recruitment of osteoblast precursor cells rather than the enhancement of the activity of existing osteoblasts.

It is found that osteoblast precursors are the major target cells for the anabolic effect of PGE₂ and that its action in these cells is mediated by EP₄. The EP₄
15 receptor subtype is believed to be the major receptor which mediates the effects of PGE₂ in rat bone tissue. Induction of EP₄ by PGE₂ further supports its biological role in the bone tissue and points to a mechanism of autoamplification of PGE action.
20 incorporated by reference herein in its entirety.

Despite the scientific information that is known on prostaglandins and prostaglandin receptor subtypes, it has previously neither been taught nor suggested that agonists of the EP₄ receptor subtype would be useful for stimulating bone formation.

25 Methods Of Stimulating Bone Formation

The present invention relates to methods for stimulating bone formation, i.e. osteogenesis, in a mammal comprising administering to a mammal in need thereof a therapeutically effective amount of an EP₄ receptor subtype agonist.

The methods and compositions of the present invention are useful for
30 both treating and reducing the risk of disease states or conditions associated with abnormal bone resorption. Such disease states or conditions include, but are not limited to, osteoporosis, glucocorticoid induced osteoporosis, Paget's disease, abnormally increased bone turnover, periodontal disease, tooth loss, bone fractures,

rheumatoid arthritis, periprosthetic osteolysis, osteogenesis imperfecta, metastatic bone disease, hypercalcemia of malignancy, and multiple myeloma.

In further embodiments, the methods comprise administering a therapeutically effective amount of an EP₄ receptor subtype agonist and a
5 bisphosphonate active. Both concurrent and sequential administration of the EP₄ receptor subtype agonist and the bisphosphonate active are deemed within the scope of the present invention. With sequential administration, the agonist and the bisphosphonate can be administered in either order. In a subclass of sequential
10 administration the agonist and bisphosphonate are typically administered within the same 24 hour period. In yet a further subclass, the agonist and bisphosphonate are typically administered within about 4 hours of each other.

The term "therapeutically effective amount", as used herein, means that amount of the EP₄ receptor subtype agonist, or other actives of the present invention, that will elicit the desired therapeutic effect or response or provide the desired benefit
15 when administered in accordance with the desired treatment regimen. A preferred therapeutically effective amount is a bone formation stimulating amount.

"Pharmaceutically acceptable" as used herein, means generally suitable for administration to a mammal, including humans, from a toxicity or safety standpoint.

20 In the present invention, the agonist is typically administered for a sufficient period of time until the desired therapeutic effect is achieved. The term "until the desired therapeutic effect is achieved", as used herein, means that the therapeutic agent or agents are continuously administered, according to the dosing schedule chosen, up to the time that the clinical or medical effect sought for the
25 disease or condition being mediated is observed by the clinician or researcher. For methods of treatment of the present invention, the compounds are continuously administered until the desired change in bone mass or structure is observed. In such instances, achieving an increase in bone mass or a replacement of abnormal bone structure with normal bone structure are the desired objectives. For methods of
30 reducing the risk of a disease state or condition, the compounds are continuously administered for as long as necessary to prevent the undesired condition. In such instances, maintenance of bone mass density is often the objective.

Nonlimiting examples of administration periods can range from about 2 weeks to the remaining lifespan of the mammal. For humans, administration

periods can range from about 2 weeks to the remaining lifespan of the human, preferably from about 2 weeks to about 20 years, more preferably from about 1 month to about 20 years, more preferably from about 6 months to about 10 years, and most preferably from about 1 year to about 10 years.

5

Methods Of Identifying Agonists Of The EP₄ Receptor Subtype

The present invention also relates to methods for identifying compounds useful as agonists of the EP₄ receptor subtype. Compounds so identified are useful for stimulating bone formation.

10

The present invention relates to a method for identifying compounds which agonize an EP₄ receptor subtype comprising:

- a). contacting a putative agonist of an EP₄ receptor subtype with a cell culture; and
- b). determining the agonist activity by comparing the agonist activity from the cell culture so contacted (i.e. the cell culture contacted with said putative agonist) with a cell culture not contacted with said putative agonist.

15

The present invention also relates to a method for identifying a compound which agonizes an EP₄ receptor subtype comprising:

- a). contacting a putative agonist of an EP₄ receptor subtype with an EP₄ receptor; and
- b). determining the agonist activity by comparing the agonist activity from the EP₄ receptor so contacted (i.e. the EP₄ receptor contacted with said putative agonist) with the agonist activity from an EP₄ receptor not contacted with said putative agonist.

20

Compositions Of The Present Invention

The pharmaceutical compositions of the present invention comprise a therapeutically effective amount of an EP₄ receptor agonist.

These compositions can further comprise a pharmaceutically-acceptable carrier. In further embodiments these compositions also comprise a bisphosphonate active.

25

EP₄ Receptor Subtype Agonist

The methods and compositions of the present invention comprise an EP₄ receptor subtype agonist.

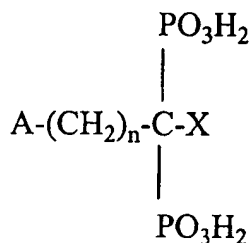
The term "agonist" as used herein, is used in its standard meaning to mean a chemical substance that can interact with a receptor and initiate a physiological or pharmacological response characteristic of that receptor.

The agonists useful herein generally have an EC₅₀ value from about 0.1 nM to about 100 microM, although agonists with activities outside this range can be useful depending upon the dosage and route of administration. In a subclass of the present invention, the agonists have an EC₅₀ value of from about 0.01 microM to about 10 microM. In a further subclass of the present invention, the agonists have an EC₅₀ value of from about 0.1 microM to about 10 microM. EC₅₀ is a common measure of agonist activity well known to those of ordinary skill in the art and is defined as the concentration or dose of an agonist that is needed to produce half, i.e. 50%, of the maximal effect. See also, Goodman and Gilman's, *The Pharmacologic Basis of Therapeutics*, 9th edition, 1996, chapter 2, E. M. Ross, *Pharmacodynamics, Mechanisms of Drug Action and the Relationship Between Drug Concentration and Effect*, which is incorporated by reference herein in its entirety.

Nonlimiting examples of agonists are selected from the group consisting of prostaglandin E₁, prostaglandin E₂, misoprostal, 19-hydroxy prostaglandin E₂, 9-oxo-8-phenyl-8-(5-phenylpentyl)decanoic acid, 8-acetyl-8-phenyl-13-phenoxytridecanoic acid, and the pharmaceutically acceptable salts thereof, and mixtures thereof.

Bisphosphonates

The methods and compositions of the present invention, can further comprise a bisphosphonate active. The bisphosphonates of the present invention correspond to the chemical formula



wherein n is an integer from 0 to 7 and wherein A and X are independently selected from the group consisting of H, OH, halogen, NH₂, SH, phenyl, C1-C30 alkyl, C3-C30 branched or cycloalkyl, C1-C30 substituted alkyl, C1-C10 alkyl substituted NH₂, C3-C10 branched or cycloalkyl substituted NH₂, C1-C10 dialkyl substituted NH₂,
5 C3-C10 branched or cycloalkyl disubstituted NH₂, C1-C10 alkoxy, C1-C10 alkyl substituted thio, thiophenyl, halophenylthio, C1-C10 alkyl substituted phenyl, pyridyl, furanyl, pyrrolidinyl, imidazolyl, imidazopyridinyl, and benzyl, such that both A and X are not selected from H or OH when n is 0; or A and X are taken together with the carbon atom or atoms to which they are attached to form a C3-C10 ring.

10 In the foregoing chemical formula, the alkyl groups can be straight, branched, or cyclic, provided that sufficient atoms are selected for the chemical formula. The C1-C30 substituted alkyl can include a wide variety of substituents, nonlimiting examples which include those selected from the group consisting of phenyl, pyridyl, furanyl, pyrrolidinyl, imidazolyl, NH₂, C1-C10 alkyl or dialkyl
15 substituted NH₂, OH, SH, and C1-C10 alkoxy.

The foregoing chemical formula is also intended to encompass complex carbocyclic, aromatic and hetero atom structures for the A and/or X substituents, nonlimiting examples of which include naphthyl, quinolyl, isoquinolyl, adamantyl, and chlorophenylthio.

20 A non-limiting class of structures useful in the instant invention are those in which A is selected from the group consisting of H, OH, and halogen, X is selected from the group consisting of C1-C30 alkyl, C1-C30 substituted alkyl, halogen, and C1-C10 alkyl or phenyl substituted thio, and n is 0.

A non-limiting subclass of structures useful in the instant invention are
25 those in which A is selected from the group consisting of H, OH, and Cl, X is selected from the group consisting of C1-C30 alkyl, C1-C30 substituted alkyl, Cl, and chlorophenylthio, and n is 0.

A non-limiting example of the subclass of structures useful in the instant invention is when A is OH and X is a 3-aminopropyl moiety, and n is 0, so that
30 the resulting compound is a 4-amino-1-hydroxybutylidene-1,1-bisphosphonate, i.e. alendronate.

Pharmaceutically acceptable salts and derivatives of the bisphosphonates are also useful herein. Nonlimiting examples of salts include those selected from the group consisting alkali metal, alkaline metal, ammonium, and
35 mono-, di, tri-, or tetra-C1-C30-alkyl-substituted ammonium. Preferred salts are those

selected from the group consisting of sodium, potassium, calcium, magnesium, and ammonium salts. Nonlimiting examples of derivatives include those selected from the group consisting of esters, hydrates, and amides.

It should be noted that the terms "bisphosphonate" and "bisphosphonates", as used herein in referring to the therapeutic agents of the present invention are meant to also encompass diphosphonates, biphosphonic acids, and diphosphonic acids, as well as salts and derivatives of these materials. The use of a specific nomenclature in referring to the bisphosphonate or bisphosphonates is not meant to limit the scope of the present invention, unless specifically indicated.

Because of the mixed nomenclature currently in use by those of ordinary skill in the art, reference to a specific weight or percentage of a bisphosphonate compound in the present invention is on an acid active weight basis, unless indicated otherwise herein. For example, the phrase "about 5 mg of a bisphosphonate selected from the group consisting of alendronate, pharmaceutically acceptable salts thereof, and mixtures thereof, on an alendronic acid active weight basis" means that the amount of the bisphosphonate compound selected is calculated based on 5 mg of alendronic acid. For other bisphosphonates, the amount of bisphosphonate is calculated based on the corresponding bisphosphonic acid.

Nonlimiting examples of bisphosphonates useful herein include the following:

Alendronic acid, 4-amino-1-hydroxybutylidene-1,1-bisphosphonic acid.

Alendronate (also known as alendronate sodium or alendronate monosodium trihydrate), 4-amino-1-hydroxybutylidene-1,1-bisphosphonic acid monosodium trihydrate.

Alendronic acid and alendronate are described in U.S. Patents 4,922,007, to Kieczkowski et al., issued May 1, 1990; 5,019,651, to Kieczkowski et al., issued May 28, 1991; 5,510,517, to Dauer et al., issued April 23, 1996; 5,648,491, to Dauer et al., issued July 15, 1997, all of which are incorporated by reference herein in their entirety.

Cycloheptylaminomethylene-1,1-bisphosphonic acid, YM 175, Yamanouchi (cimadronate), as described in U.S. Patent 4,970,335, to Isomura et al., issued November 13, 1990, which is incorporated by reference herein in its entirety.

1,1-dichloromethylene-1,1-diphosphonic acid (clodronic acid), and the disodium salt (clodronate, Procter and Gamble), are described in Belgium Patent 672,205 (1966) and *J. Org. Chem* 32, 4111 (1967), both of which are incorporated by reference herein in their entirety.

5 1-hydroxy-3-(1-pyrrolidinyl)-propylidene-1,1-bisphosphonic acid (EB-1053).

1-hydroxyethane-1,1-diphosphonic acid (etidronic acid).

10 1-hydroxy-3-(N-methyl-N-pentylamino)propylidene-1,1-bisphosphonic acid, also known as BM-210955, Boehringer-Mannheim (ibandronate), is described in U.S. Patent No. 4,927,814, issued May 22, 1990, which is incorporated by reference herein in its entirety.

6-amino-1-hydroxyhexylidene-1,1-bisphosphonic acid (neridronate).

15 3-(dimethylamino)-1-hydroxypropylidene-1,1-bisphosphonic acid (olpadronate).

3-amino-1-hydroxypropylidene-1,1-bisphosphonic acid (pamidronate).

20 [2-(2-pyridinyl)ethylidene]-1,1-bisphosphonic acid (piridronate) is described in U.S. Patent No. 4,761,406, which is incorporated by reference in its entirety.

1-hydroxy-2-(3-pyridinyl)-ethylidene-1,1-bisphosphonic acid (risedronate).

25 (4-chlorophenyl)thiomethane-1,1-disphosphonic acid (tiludronate) as described in U.S. Patent 4,876,248, to Breliere et al., October 24, 1989, which is incorporated by reference herein in its entirety.

1-hydroxy-2-(1^H-imidazol-1-yl)ethylidene-1,1-bisphosphonic acid (zolendronate).

30 A non-limiting class of bisphosphonates useful in the instant invention are selected from the group consisting of alendronate, cimadronate, clodronate, tiludronate, etidronate, ibandronate, neridronate, olpandronate, risedronate, piridronate, pamidronate, zolendronate, pharmaceutically acceptable salts thereof, and mixtures thereof.

A non-limiting subclass of the above-mentioned class in the instant case is selected from the group consisting of alendronate, pharmaceutically acceptable salts thereof, and mixtures thereof.

5 A non-limiting example of the subclass is alendronate monosodium trihydrate.

Other Components Of The Pharmaceutical Compositions

The EP₄ receptor subtype agonists, and in further embodiments the bisphosphonate actives and any other additional actives are typically administered in admixture with suitable pharmaceutically acceptable diluents, excipients, or carriers, collectively referred to herein as "carrier materials", suitably selected with respect to the mode of administration. Nonlimiting examples of product forms include tablets, capsules, elixirs, syrups, powders, suppositories, nasal sprays, liquids for ocular administration, formulations for transdermal administration, and the like, consistent with conventional pharmaceutical practices. For example, for oral administration in the form of a tablet, capsule, or powder, the active ingredient can be combined with an oral, non-toxic, pharmaceutically acceptable inert carrier such as lactose, starch, sucrose, glucose, methyl cellulose, magnesium stearate, mannitol, sorbitol, croscarmellose sodium and the like. For oral administration in liquid form, e.g.,
10 elixirs and syrups, the oral drug components can be combined with any oral, non-toxic, pharmaceutically acceptable inert carrier such as ethanol, glycerol, water and the like. Moreover, when desired or necessary, suitable binders, lubricants, disintegrating agents and coloring agents can also be incorporated. Suitable binders can include starch, gelatin, natural sugars such a glucose, anhydrous lactose, free-flow
15 lactose, beta-lactose, and corn sweeteners, natural and synthetic gums, such as acacia, guar, tragacanth or sodium alginate, carboxymethyl cellulose, polyethylene glycol, waxes, and the like. Lubricants used in these dosage forms include sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride and the like. An example of a tablet formulation is that described in U.S.
20 Patent No. 5,358,941, to Bechard et al, issued October 25, 1994, which is incorporated by reference herein in its entirety. The compounds used in the present method can also be coupled with soluble polymers as targetable drug carriers. Such polymers can include polyvinylpyrrolidone, pyran copolymer, polyhydroxypropyl-methacrylamide, and the like.

35

The following Examples are presented to better illustrate the invention.

EXAMPLES

5 1. Animal Procedures:

For mRNA localization experiments, 5-week old Sprague-Dawley rats (Charles River) are euthanized by CO₂, their tibiae and calvariae are excised, cleaned of soft tissues and frozen immediately in liquid nitrogen. For EP₄ regulation experiments, 6-week old rats are given a single injection of either vehicle (7% ethanol in sterile water) or an anabolic dose of PGE₂ (Cayman Chemical, Ann Arbor, MI), 3-10 6 mg/kg in the same vehicle) intraperitoneally. Animals are euthanized at several time points post-injection and their tibiae and calvariae, as well as samples from lung and kidney tissues are frozen in liquid nitrogen.

15 2. Cell Cultures

RP-1 periosteal cells are spontaneously immortalized from primary cultures of periosteal cells from tibiae of 4-week old Sprague-Dawley rats and are cultured in DMEM (BRL, Gaithersburg, MD) with 10 % fetal bovine serum (JRH Biosciences, Lenexa, KS). These cells do not express osteoblastic phenotypic markers in early culture, but upon confluence, express type I collagen, alkaline phosphatase and osteocalcin and produce mineralized extracellular matrix.

RCT-1 and RCT-3 are clonal cell lines immortalized by SV-40 large T antigen from cells released from fetal rat calvaria by a combination collagenase/hyaluronidase digestion. RCT-1 cells, derived from cells released during 25 the first 10 minutes of digestion (fraction I), are cultured in RPMI 1640 medium (BRL) with 10% fetal bovine serum and 0.4 mg/ml G418 (BRL). These cells differentiate and express osteoblastic features upon retinoic acid treatment. RCT-3 cells, immortalized from osteoblast-enriched fraction III cells, are cultured in F-12 medium (BRL) with 5% Fetal bovine serum and 0.4 mg/ml G418. TRAB-11 cells are 30 also immortalized by SV40 large T antigen from adult rat tibia and are cultured in RPMI 1640 medium with 10% FBS and 0.4 mg/ml G418. ROS 17/2.8 rat osteosarcoma cells are cultured in F-12 containing 5% FBS. Osteoblast-enriched (fraction III) primary fetal rat calvaria cells are obtained by collagenase/hyaluronidase digestion of calvariae of 19 day-old rat fetuses. See Rodan et al., *Growth stimulation*

of rat calvaria osteoblastic cells by acidic FGF, *Endocrinology*, 121, 1919-1923 (1987), which is incorporated by reference herein in its entirety. Cells are released during 30-50 minutes digestion (fraction III) and are cultured in F-12 medium containing 5% FBS.

- 5 P815 (mouse mastocytoma) cells, cultured in Eagles MEM with 10% FBS, and NRK (normal rat kidney fibroblasts) cells, cultured in DMEM with 10% FBS, are used as positive and negative controls for the expression of EP₄, respectively. See Abramovitz et al., *Human prostanoid receptors: cloning and characterization*. In: Samulesson B. et al. ed) *Advances in prostaglandin, Thrombosnes and leukotriene research*, vol. 23, pp. 499-504 (1995) and de Larco et al., *Epithelioid and fibroblastic rat kidney cell clones: EGF receptors and the effect of mouse sarcoma virus transformation*, *Cell Physiol.*, 94, 335-342 (1978), which are both incorporated by reference herein in their entirety.

15 3. Northern Blot Analysis:

- Total RNA is extracted from the tibial metaphysis or diaphysis and calvaria using a guanidinium isothiocyanate-phenol-chloroform method after pulverizing frozen bone samples by a tissue homogenizer. See P. Chomczynski et al., *Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction.*, *Analyt Biochem*, 162, 156-159 (1987), which is incorporated by reference herein in its entirety. RNA samples (20 mg) are separated on 0.9% agarose/formaldehyde gels and transferred onto nylon membranes (Boehringer Mannheim, Germany). Membranes are prehybridized in Hybrisol I (Oncor, Gaithersburg, MD) and 0.5 mg/ml sonicated salmon sperm DNA (Boehringer) at 20 42°C for 3 hours and are hybridized at 42°C with rat EP₂ and mouse EP₄ cDNA probes labeled with [³²P]-dCTP (Amersham, Buckinghamshire, UK) by random priming using the rediprime kit (Amersham). After hybridization, membranes are washed 4 times in 2xSSC + 0.1% SDS at room temperature for a total of 1 hour and once with 0.2xSSC + 0.1% SDS at 55°C for 1 hour and then exposed to Kodak XAR 25 2 film at -70°C using intensifying screens. After developing the films, bound probes are removed twice with 0.1% SDS at 80°C and membranes are hybridized with a 30 human GAPDH (Glyceraldehyde 3-Phosphate Dehydrogenase) cDNA probe (purchased from Clontech, Palo Alto, CA) for loading control.

4. In-Situ Hybridization:

Frozen tibiae are sectioned coronally at 7 mm thickness and sections are mounted on charged slides (Probe On Plus, Fisher Scientific, Springfield, NJ) and are kept at -70°C until hybridization. cRNA probes are labeled with ³⁵S-UTPgS (ICN, Costa Mesa, CA) using a Riboprobe II kit (Promega Madison, WI).
Hybridization is performed overnight at 50° C. See M. Weinreb et al., *Different pattern of alkaline phosphatase, osteopontin and osteocalcin expression in developing rat bone visualized by in-situ hybridization*, *J. Bone Miner Res.*, 5, 831-842 (1990) and D. Shinar et al., *Expression of alphav and beta3 integrin subunits in rat osteoclasts in situ*, *J. Bone Miner. Res.*, 8, 403-414 (1993), which are both incorporated by reference herein in their entirety. Following hybridization and washing, sections are dipped in Ilford K5 emulsion diluted 2:1 with 6% glycerol in water at 42° C and exposed in darkness at 4° C for 12-14 days. Slides are developed in Kodak D-19 diluted 1:1 with water at 15°, fixed, washed in distilled water and mounted with glycerol-gelatin (Sigma) after hematoxylin staining. Stained sections are viewed under the microscope (Olympus, Hamburg, Germany), using either bright-field or dark-field optics.

5. Expression Of EP₄ In Osteoblastic Cell Lines And In Bone Tissue.

The expression of EP₄ and EP₂ mRNA is examined in various bone derived cells including osteoblast-enriched primary rat calvaria cells, immortalized osteoblastic cell lines from fetal rat calvaria or from adult rat tibia and an osteoblastic osteosarcoma cell line. Most of the osteoblastic cells and cell lines show significant amounts of 3.8 kb EP₄ mRNA, except for the rat osteosarcoma cell line ROS 17/2.8. Consistent with this finding, in ROS 17/2.8 cells PGE₂ has no effect on intracellular cAMP, which is markedly induced in RCT-3 and TRAB-11 cells. Treatment of RCT-1 cells with retinoic acid, which promotes their differentiation, reduces the levels of EP₄ mRNA. NRK fibroblasts do not express EP₄ mRNA, while P815 mastocytoma cells, used as positive controls, express large amounts of EP₄ mRNA. In contrast to EP₄ mRNA, none of the osteoblastic cells and cell lines express detectable amounts of EP₂ mRNA in total RNA samples. Expression of EP₄ mRNA in osteoblastic cells, EP₄ is also expressed in total RNA isolated from tibiae and calvariae of 5-week-old rats. In contrast, no EP₂ mRNA is found in RNA from tibial shafts.

6. PGE₂ Induces The Expression Of EP₄ mRNA in RP-1 Periosteal Cells And In Adult Rat Tibiae

PGE₂ enhances its own production via upregulation of cyclooxygenase 2 expression in osteoblasts and in bone tissue thus autoamplifying its own effects. PGE₂ also increases the levels of EP₄ mRNA. RP-1 cells are immortalized from a primary culture of adult rat tibia periosteum is examined. These cells express osteoblast phenotypic markers upon confluence and form mineralized bone matrix when implanted in nude mice. Similar to the other osteoblastic cells examined, RP-1 periosteal cells express a 3.8 kb EP₄ transcript. Treatment with PGE₂ (10⁻⁶ M) rapidly increases EP₄ mRNA levels peaking at 2 hours after treatment. PGE₂ has no effect on EP₄ mRNA levels in the more differentiated RCT-3 cells pointing to cell-type specific regulation of EP₄ expression by PGE₂. EP₂ mRNA is not expressed in RP-1 cells before or after treatment with PGE₂.

To examine if PGE₂ regulates EP₄ mRNA levels *in vivo* in bone tissue, five-week-old male rats are injected with PGE₂ (3 - 6 mg/Kg). Systemic administration of PGE₂ rapidly increased EP₄ mRNA levels in the tibial diaphysis peaking at 2 h after injection. A similar effect of PGE₂ on EP₄ mRNA is observed in the tibial metaphysis and in calvaria. PGE₂ induces EP₄ mRNA levels *in vitro* in osteogenic periosteal cells and *in vivo* in bone tissue in a cell type-specific and tissue-specific manner. PGE₂ does not induce EP₂ mRNA in RP-1 cells nor in bone tissue.

7. Localization of EP₄ mRNA expression in bone tissue

In situ hybridization is used in order to localize cells expressing EP₄ in bone. In control experiment (vehicle-injected) rats, low expression of EP₄ is detected in bone marrow cells. Administration of a single anabolic dose of PGE₂ increases the expression of EP₄ in bone marrow cells. The distribution of silver grains over the bone marrow is not uniform and occurs in clumps or patches in many areas of the metaphysis. Within the tibial metaphysis, EP₄ expression is restricted to the secondary spongiosa area and is not seen in the primary spongiosa. Hybridization of similar sections with a sense probe (negative control) does not show any signal.

EP₄ is expressed in osteoblastic cells *in vitro* and in bone marrow cells *in vivo*, and is upregulated by its ligand, PGE₂.

8. Agonists Of the Present Invention

Using standard methods for measuring agonist activity, the following compounds are evaluated in cell cultures and in EP₄ receptor cell-free systems to determine the agonist activity of the compounds in terms of their EC₅₀ value: prostaglandin E₁, prostaglandin E₂, misoprostal, 19-hydroxy prostaglandin E₂, 9-oxo-
 5 8-phenyl-8-(5-phenylpentyl)decanoic acid, and 8-acetyl-8-phenyl-13-phenoxytridecanoic acid.

9. Pharmaceutical tablets

Pharmaceutical tablets are prepared using standard mixing and
 10 formation techniques.

Tablets containing about 1 to 100 mg of an EP₄ receptor subtype agonist are prepared using the following relative weights of ingredients.

<u>Ingredient</u>	<u>Per Tablet</u>
15 EP ₄ Receptor Subtype Agonist	1 to 100 mg
Anhydrous Lactose, NF	71.32 mg
Magnesium Stearate, NF	1.0 mg
Croscarmellose Sodium, NF	2.0 mg
20 Microcrystalline Cellulose, NF	QS 200 mg

The resulting tablets are useful for administration in accordance with the methods of the present invention for stimulating bone formation.

In further embodiments, tablets are prepared that also contain 5 or 10
 25 mg of a bisphosphonate active, on a bisphosphonic acid active basis, of a bisphosphonate selected from the group consisting of alendronate, clodronate, tiludronate, etidronate, ibandronate, neridronate, olpandronate, risedronate, piridronate, pamidronate, zolendronate, and pharmaceutically acceptable salts thereof.

10. Liquid Formulation.

Liquid formulations are prepared using standard mixing techniques.
 A liquid formulation containing about 1 to about 100 mg of an EP₄
 35 receptor subtype agonist is prepared using the following relative weights of ingredients.

	<u>Ingredient</u>	<u>Weight</u>
	EP ₄ Receptor Subtype Agonist	1-100 mg
5	Sodium Propylparaben	22.5 mg
	Sodium Butylparaben	7.5 mg
	Sodium Citrate Dihydrate	1500 mg
	Citric Acid Anhydrous	56.25 mg
	Sodium Saccharin	7.5 mg
10	Water	qs 75 mL
	1 N Sodium Hydroxide (aq)	qs pH 6.75

The resulting liquid formulation is useful for administration for stimulating bone formation.

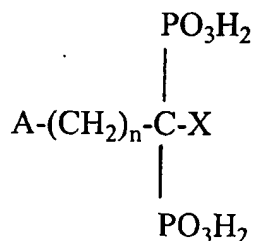
- 15 In further embodiments solutions are prepared also containing 5 or 10 mg of a bisphosphonate active, on a bisphosphonic acid active basis, of a bisphosphonate selected from the group consisting of alendronate cimadronate, clodronate, tiludronate, etidronate, ibandronate, neridronate, olpandronate, risedronate, piridronate, pamidronate, zolendronate, and pharmaceutically acceptable
- 20 salts thereof.

WHAT IS CLAIMED IS:

1. A method for stimulating bone formation in a mammal in need thereof comprising administering to said mammal a therapeutically effective amount of an EP₄ receptor subtype agonist.
5
2. A method according to Claim 1 wherein said mammal is a human.
3. A method for treating or reducing the risk of contracting a disease state or condition in a mammal in need of such treatment or risk reduction, comprising administering to said mammal a therapeutically effective amount of an EP₄ receptor subtype agonist.
10
4. A method according to Claim 3 wherein said mammal is a human.
15
5. A method according to Claim 4 wherein said disease state or condition is selected from the group consisting of osteoporosis, glucocorticoid induced osteoporosis, Paget's disease, abnormally increased bone turnover, periodontal disease, tooth loss, bone fractures, rheumatoid arthritis, periprosthetic osteolysis, osteogenesis imperfecta, metastatic bone disease, hypercalcemia of malignancy, and multiple myeloma.
20
6. A method according to Claim 5 wherein said disease state or condition is selected from the group consisting of osteoporosis, glucocorticoid induced osteoporosis, and periodontal disease.
25
7. A method according to Claim 1 wherein said agonist is selected from the group consisting of PGE₁, PGE₂, misoprostal, 19-hydroxy prostaglandin E₂, 9-oxo-8-phenyl-8-(5-phenylpentyl)decanoic acid, 8-acetyl-8-phenyl-13-phenoxytridecanoic acid, and the pharmaceutically acceptable salts thereof, and mixtures thereof.
30

8. A method for stimulating bone formation in a mammal in need thereof comprising administering to said mammal a therapeutically effective amount of an EP₄ receptor subtype agonist and a bisphosphonate active.

9. A method according to Claim 8 wherein said bisphosphonate active corresponds to the chemical structure



wherein n is an integer from 0 to 7 and wherein A and X are independently selected from the group consisting of H, OH, halogen, NH₂, SH, phenyl, C1-C30 alkyl, C3-C30 branched or cycloalkyl, C1-C30 substituted alkyl, C1-C10 alkyl substituted NH₂, C3-C10 branched or cycloalkyl substituted NH₂, C1-C10 dialkyl substituted NH₂, C1-C10 alkoxy, C1-C10 alkyl substituted thio, thiophenyl, halophenylthio, C1-C10 alkyl substituted phenyl, pyridyl, furanyl, pyrrolidinyl, imidazolyl, imidazopyridinyl, and benzyl; or A and X are taken together with the carbon atom or atoms to which they are attached to form a C3-C10 ring; and provided that when n is 0, A and X are not selected from the group consisting of H and OH; and the pharmaceutically acceptable salts thereof.

10. A method according to Claim 8 wherein said bisphosphonate is selected from the group consisting of alendronate, cimadronate, clodronate, tiludronate, etidronate, ibandronate, neridronate, olpandronate, risedronate, piridronate, pamidronate, zolendronate, pharmaceutically acceptable salts thereof, and mixtures thereof.

11. A method according to Claim 10 wherein said bisphosphonate is alendronate, pharmaceutically acceptable salts thereof, and mixtures thereof.

12. A method according to Claim 11 wherein said bisphosphonate is alendronate monosodium trihydrate.

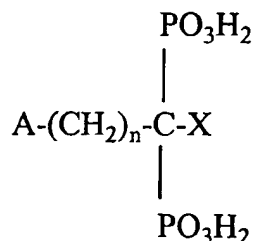
13. A pharmaceutical composition comprising a therapeutically effective amount of an EP₄ receptor subtype agonist.

14. A pharmaceutical composition according to Claim 13 which further comprises a pharmaceutically acceptable carrier.

15. A pharmaceutical composition according to Claim 14 wherein said agonist has an EC₅₀ value from about 0.1 nanoM to about 100 microM.

16. A pharmaceutical composition according to Claim 12 which further comprises a therapeutically effective amount of a bisphosphonate active.

17. A pharmaceutical composition according to Claim 16 wherein said bisphosphonate active corresponds to the chemical structure



wherein n is an integer from 0 to 7 and wherein A and X are independently selected from the group consisting of H, OH, halogen, NH₂, SH, phenyl, C1-C30 alkyl, C3-C30 branched or cycloalkyl, C1-C30 substituted alkyl, C1-C10 alkyl substituted NH₂, C3-C10 branched or cycloalkyl substituted NH₂, C1-C10 dialkyl substituted NH₂, C1-C10 alkoxy, C1-C10 alkyl substituted thio, thiophenyl, halophenylthio, C1-C10 alkyl substituted phenyl, pyridyl, furanyl, pyrrolidinyl, imidazolyl, imidazopyridinyl, and benzyl; or A and X are taken together with the carbon atom or atoms to which they are attached to form a C3-C10 ring; and provided that when n is 0, A and X are

not selected from the group consisting of H and OH; and the pharmaceutically acceptable salts thereof.

5 18. A pharmaceutical composition according to Claim 16 wherein said bisphosphonate is selected from the group consisting of alendronate, cimidronate, clodronate, tiludronate, etidronate, ibandronate, neridronate, olpandronate, risedronate, piridronate, pamidronate, zolendronate, pharmaceutically acceptable salts thereof, and mixtures thereof.

10 19. A pharmaceutical composition according to Claim 18 wherein said bisphosphonate is alendronate, pharmaceutically acceptable salts thereof, and mixtures thereof.

15 20. A pharmaceutical composition according to Claim 19 wherein said bisphosphonate is alendronate monosodium trihydrate.

 21. A method for identifying a compound which agonizes an EP₄ receptor subtype comprising:

- 20 a). contacting a putative agonist of an EP₄ receptor subtype with a cell culture; and
- b). determining the agonist activity of said putative agonist with a cell culture not contacted with said putative agonist.

25 22. A method for identifying a compound which agonizes an EP₄ receptor subtype comprising:

- a). contacting a putative agonist of an EP₄ receptor subtype with an EP₄ receptor; and
- b). determining the agonist activity of said putative agonist with an EP₄ receptor not contacted with said putative agonist.

30

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US99/23757

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/66, 31/19

US CL :514/102, 108, 573

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/102, 108, 573

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,616,571 A (DAIFOTIS et al.) 01 April 1997 see entire document.	1-22
A	US 5,730,715 A (SAGE, JR. et al.) 24 March 1998 see entire document.	1-22
A,P	US 5,892,099 A (MARUYAMA et al.) 06 April 1999, see entire document.	1-22



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
B earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

15 NOVEMBER 1999

Date of mailing of the international search report

04 FEB 2000

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